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APPLICATION NUMBER: *60/489,026*
FILING DATE: *July 22, 2003*

Certified by

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

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TITLE OF INVENTION (280 characters max)

IMPROVING PRODUCTION CHARACTERISTICS OF CATTLE

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Date of Deposit: 7/22/03

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ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages including cover sheet: 15

☐ CD(s), Number:

☒ Drawing(s) Number of Sheets: 10

☐ Other (specify):

☐ Application Data Sheet. See 37 CFR 1.76

METHOD FOR PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

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Respectfully submitted, Date: 7/22/03

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Application for Letters Patent

IMPROVING PRODUCTION CHARACTERISTICS OF CATTLE

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IMPROVING PRODUCTION CHARACTERISTICS OF CATTLE

FIELD OF THE INVENTION

The present invention relates generally to methods of selective breeding and management of beef cattle, and particularly to predicting the production of high quality beef.

BACKGROUND

Weight gain by a livestock animal during its growth and development typically follows a tri-phasic pattern that is carefully managed by commercial producers, and finishers. The efficiency of dietary caloric (feed) conversion to weight gain during an increment of time varies during three growth phases; a first phase of growth comprises that portion of a livestock animals life from birth to weaning, and is not paid much heed by commercial feeding and finishing operators.

A second growth phase comprises that portion of a livestock animal's life from weaning to attainment of musculo-skeletal maturity. As feed conversation efficiency is low during this phase, livestock producers usually restrict caloric intake. This has the effect of causing this phase to be prolonged, but also typically results in animals with larger frames, the primary aim of dietary management during this phase. During the second growth phase weight gain is associated with skeletal mass and muscle mass accumulation primarily.

During a third growth phase, after an animal has attained musculo-skeletal maturity, the efficiency of feed conversion is reduced, such that it requires more feed to increase an animal's weight. For example, with cattle, during the second phase of growth, a typical steer could convert 5 to 6 pounds of feed into one pound of weight gain. Upon entering the third phase, feed conversion efficiency typically decreases, such that 7 up to 10 or

more pounds of feed are required to produce one pound of gain. During the third phase livestock feeders significantly increase the caloric content of animals' rations. During the third growth phase weight gain is associated with fat accumulation primarily.

In cattle production, the goal of breeding and herd management programs is to increase the amount of high value cuts of meat in the finished animal. A measurement associated with higher value carcasses is rib eye area (REA) size. However, using current methods of breeding and culling based on parental animal frame size, it is not possible to realize the full genetic potential of individual animals in terms of high value meat production.

Insulin-like growth factor 2 (IGF2) is a 67 amino acid peptide hormone having multiple phenotypic effects on cellular growth and metabolism. *IGF2* comprises 10 exons in pig (Amarger et al. 2002) and human (McLaren and Montgomery 1999), while sheep *IGF2* has 9 exons (Figure 1) (Ohlsen et al. 1994). No equivalent to human exon 2 has been found in sheep (Ohlsen et al. 1994). The mouse and rat *IGF2* have six exons and two pseudo or non-coding exons (Ohlsen et al. 1994) (Figure 1).

IGF2 was previously thought to function primarily as a fetal and neonatal growth factor (De Chiara et al. 1990, 1991, Rotwein and Hall 1990, Giannoukakis et al. 1993). However, a role for *IGF2* during the postnatal development of lean meat carcass content of pigs (i.e. ham size) has recently been demonstrated (Jeon et al. 1999, Nezer et al. 1999, Amarger et al. 2002). In several species such as human, mice and sheep, expression of *IGF2* appears to be subject to genomic imprinting. The paternal copy is constitutively expressed, while expression of the maternal copy varies during development.

DESCRIPTION OF THE DRAWINGS:

While the invention is claimed in the preceding portions hereof, preferred embodiments are provided in the accompanying detailed description which may be best understood in conjunction with the accompanying diagrams, where like parts in each diagram are labeled with like numbers and where

Figure 1: A comparison of the genomic organization of the pig, sheep, human, rat, mouse, and cow *IGF2* genes (Adapted from Ohlsen et al. 1994, Amarger et al. 2002). The numbered boxes represent the *IGF2* exons. Open and solid boxes indicate untranslated and translated exons respectively. ϕ1 and ϕ2 are the two-psuedo exons identified in the mouse *IGF2* gene (Rotwein and Hall 1990). Promoters are indicated by P1 - P4.

Figure 2: A gene structure comparison of the 7 different *IGF2* transcripts found in the different tissues of an 18 month-old steer. The black exons are the transcribed exons in each mRNA. Note that only exons 8-10 are translated into protein and are present in every transcript.

Figure 3: Photograph of an ethidium bromide stained agarose gel (3%) of an embryo transfer family from a heterozygous (C/T) Charolais sire and homozygous (C/C) Limousin dam. Four calves are heterozygous for the T allele and the other four calves are homozygous for the C allele. The C allele is 185 bp and the T allele is 118 and 68 bp. The 32 bp product is not shown.

Figure 4: A linkage map showing the location of the microsatellites used to map IGF2 to the telomeric end of BTA 29.

Figure 5: Photograph of an ethidium bromide stained agarose gel (3%) of a possible informative family for the exon 2 SNP. Calf M444 in lane 2 is heterozygous (C/T), as is his dam, cow 491 (C/T) in lane 3. For this family to be informative, the sire would have to be homozygous C/C as is the animal in lane 1 (or T/T) to assign parental origin to the calf's alleles.

Figure 6: Photograph of an ethidium bromide stained agarose gel (3%) of the different alleles observed with the exon 2 SNP digested with *Bsr1*. To demonstrate genomic imprinting, fetal tissues would be harvested from a Holstein calf heterozygous (C/T) for

the exon 2 SNP. Expression analysis is expected to display expression of the paternal allelle. The genomic DNA would show heterozygosity (C/T) while the cDNA from the different tissues would express the paternal allele only (C or T). As the animal ages, biallelic expression (C/T) would be expected to reappear in the liver tissue but not in the kidney tissue (C or T).

Figure 7: cDNA Sequences of IGF2 Gene Exon and Introns

Table 1: Sequenced exons and introns of the cattle *IGF2* gene to date.

Table 2: The presence of the 7 different *IGF2* transcripts in each of the 15 different tissues from an 18 month-old steer as indicated by *. The transcription of the different transcripts is initiated by 1 of 4 different promoters (P1-P4).

THE INVENTION:

The following studies have revealed that a single nucleotide polymorphism (SNP) in exon 2 of *IGF2* in cattle is associated with increased rib eye area (REA) production. In addition, genetic imprinting by the mother appears to enhance the effect of the exon 2 SNP on REA size.

Materials and Methods

Animals

Gene expression studies were conducted using a crossbred beef steer slaughtered at 18 months and a 5-week-old Holstein bull calf. Fifteen tissues were collected and flash frozen in liquid nitrogen for mRNA preparation. Two sire-dam-calf Holstein families were used to study genomic imprinting. The calves were both less than a few weeks old. The 17 full-sib families of the Canadian Beef Reference Herd were used for mapping IGF2. Carcass and growth data from the offspring of these families were also used to study the effects of *IGF2* mutations.

PCR

Sheep exon boundaries (GenBank, U00659, and U00663-U00669) were used to determine the exon boundaries of a partial coding (215bp, exon 8-9) *Bos taurus* sequence (GenBank, Z68158) and (823 bp, exons 8-10) *Bos indicus* (AF283002) sequence. Primers were designed using *Bos taurus* and *Bos indicus* sequence preferentially over sheep sequence (GenBank U00663-U00668) as a template when published *IGF2* sequence from that area of the gene was available. Primers were designed to sequence all 10 exons of *IGF2* from cDNA of a crossbred steer as well as 7 exons and 1.5 introns in genomic DNA from the 5 Canadian Beef Reference Herd (CBRH) sires (Table 1).

Results

Sequencing

The complete cDNA sequences of exons represented in bovine mRNA transcripts were obtained. Genomic sequence was also obtained from the 5 sires of the CBRH families for most exons and a few introns, including intron 8 and 9 (Figure 7).

Alternate Transcripts

IGF2 mRNA was present in 15 different tissues analyzed in an 18 month-old *Bos taurus* steer (Goodall 2002). Further analysis revealed that not all cattle tissues expressed the same transcript of *IGF2* (Goodall, 2002). Alternate splicing combined with the use of alternate promoters of *IGF2* leads to the expression of several forms of *IGF2* mRNA in pigs (Amarger et al. 2002), humans (De Pagter-Holthuizen et al. 1987, 1988, Holtuizen et al. 1990), and sheep (Ohlsen et al. 1994).

Exons 8, 9 and 10, considered the coding exons (Figure 1), were present in every tissue examined from this steer suggesting that they are essential for *IGF2* function and remain part of every transcript. However different transcripts containing different 5' noncoding exons (Figure 1) were detected in several tissues (Table 2).

In total, 7 different transcripts have been identified (Figure 2), identical to those found in pigs (Amarger et al. 2002). Five of the 7 transcripts have been found in sheep (Ohlsen et al. 1994) and humans (De Pagter-Holthuizen et al. 1987, 1988, Holtuizen et al. 1990). All 7 transcripts were present in the liver of the adult steer. The 7-10 transcript was the most common transcript detected in 12 of the 15 tissues tested.

SNP's

Polymerase chain reaction conditions: The PCR reaction of 15 µl contained 1 µl of DNA template (50-100 ng), 1.5 µl of 10X PCR buffer (Invitrogen), 0.45 µl of 50 mM $MgCl_2$, 0.3 µl of 10 mM dNTPs, 0.1 µl *Taq* polymerase (5 U μl^{-1}: Invitrogen), 1 µl of each primer (10 pM μl^{-1}) and 9.6 µl of ddH_2O). The reaction began with a 4 min step at 94°C, followed by 34 cycles of 50 seconds at 94°C, 50 seconds at 64°C, and 50 seconds at 72°C. Digestion of 8 µl of each PCT product was performed with 1 µl of *Bsr*I (5 U µl-1) for 3 hours at 65°C.

Primer sequences:

Forward:	CCTCAGCCTCATCCCCTCCTTTGC
Reverse:	CTGTGCTCTATTTGCTGTGTTGTCT

Two polymorphisms (Table 1) were found in the *IGF2* gene in the 5 CBRH sires. The first SNP was located in the 3' end of intron 8 and the second in the middle of exon 2.

Mapping

The exon 2 SNP was used to linkage map the *IGF2* gene in cattle. The 217 bp fragment contained one inherent restriction site for *Bsr*I at position 32. The presence of a SNP at nucleotide 150 added an additional restriction site. After digestion with *Bsr*I the C allele consisted of 32 and 185 bp fragments and the T allele consisted of 32, 67 and 118 bp fragments. Alleles were resolved on a 3% agarose gel (Figure 3, 6). The T allele occurred with a frequency of 0.17 in the 24 purebred cattle of the parental generation.

IGF2 was found to map to the telomeric end of cattle chromosome 29, 0 cM from *ILSTS081* (LOD = 6.62). CRI-MAP generated a map order of *BMC8012 - ILSTS015 - Tyrosinase - BMC3224 - BMS764 - BMC1206 - ILSTSO81 - IGF2* (Figure 4) (in press). These data are consistent with comparative mapping data as well as our previous *in situ* hybridization mapping of *IGF2* to the distal end of cattle chromosome 29 (Schmutz et al. 1996). This sequence was entered into GenBank as accession #AY237543 and AY237544 and will be released at the time of manuscript publication.

Effects

In most of the tissues examined, the exon 2 SNP (C/T) is not transcribed and would not be expected to have any direct effect on either the structure or levels of the IGF2 protein. However, in liver, exon 2 is transcribed. Thus, mutations in exon 2 could cause changes in *IGF2* gene expression, either through changes in the stability or translational efficiency of *IGF2* mRNA, even though the exon sequences do not encode part of the final IGF2 protein. The exon 2 SNP might also causes changes in transcriptional efficiency. Whatever the mechanism, the end result is that the *IGF2* SNP functions to vary the level of IGF2 protein expression.

Preliminary analysis examining the effects of the exon 2 SNP suggested a correlation with increased REA size. REA size was corrected for sex of offspring. 108 of the offspring were either C/C or C/T. Heterozygous C/T offspring had a significantly smaller REA size than homozygous C/C offspring ($t = 3.625$, $P = 0.0004$) at 19-20 months of age. The mean area of the 93 C/C animals was 109.8 cm^2 compared to the 29 C/T animals where the mean area was 100.4 cm^2. In the 125 cattle studies, REA size was significantly correlated with the number of C alleles present in the population($r = 0.39$, $P = 0.0001$).

The parental origin of the T allele also appeared to influence the effect on REA size, consistent with genomic imprinting. There was a significant effect on REA size when the mother was the segregating parent ($t = 1.851$, $P = 0.0777$) but not when the father was the segregating parent ($t = -0.387$, $P = 0.7039$). If the T allele was inherited maternally, there was a decrease in the mean area of the REA size to 89.81cm^2 from 99.92cm^2 if the C

allele was so inherited. These results suggest that genomic imprinting is playing a role in the effect on REA size.

Although REA size was correlated with carcass weight (r =0.382, P =0.0001), there was no significant correlation between the exon 2 SNP and sex-corrected carcass weight (r =0.096, P =0.2855). This suggests that *IGF2* affects the muscle growth but not overall growth (total animal carcass weight). *IGF2* may act as a partitioning agent as the C allele increases REA size and decreases fat while the T allele decreases REA size and increases fat thereby not affecting the overall carcass weight.

The *IGF2* SNP had a significant effect on sex corrected fat (t =-1.881, P =0.0624) and sex corrected marbling (t =-1.874, P =0.0633). The 93 C/C animals had a mean fat of 9.069 and a mean marbling of 457.72 while the 29 C/T animals had a mean fat of 10.679 and a mean marbling of 484.952. This implies that *IGF2* may have an effect on fat and may function as a partitioning agent. This suggests that this SNP affects muscle size but not overall weight.

REA size was correlated to raw birth weight (r =0.188, P =0.0001) but there was no significant effect on adjusted birth weight (t = 0.127, P =0.8994). No significant correlation was noted with birth weight and the exon 2 SNP (r =0.004, P =0.9644). This suggests that the 1-3, 8-10 transcript (contains exon 2) activated by the P1 promoter does not have a primary function in fetal/neonatal growth period and therefore does not affect birth weight. It is generally believed that promoters P2-4 (Figure 1) are highly expressed in the human neonatal/fetal growth period and therefore would be the predominant fetal transcripts having affects on growth at this stage in life. However, the P1 promoter is still somewhat active during fetal growth as transcripts 1; 8-10; 1,3, 8-10; and 1-3, 8-10 have been observed in pig fetal tissue (Amarger et al. 2002).

Genomic Imprinting

The exon 2 SNP was used to determine if *IGF2* was paternally expressed in cattle as it is in mice, (De Chiara et al. 1991, Sasaki et al. 1992), rats (Ohlsson et al. 1993), humans

(Kalscheuer et al. 1993, Giannoukakis et al. 1993), pigs (Nezer et al. 1999, Jeon et al. 1999), and sheep (McLaren and Montgomery 1999, Feil et al. 1998). To test for genomic imprinting, tissues were collected from a Holstein calf (M444) heterozygous for this SNP (Figure 5). The mother and father of the calf were also genotyped to assign parent of origin to each allele in an informative family. The dam was also C/T and the sire was C/C so the T allele was of maternal origin in this 5-week-old calf. Both the maternal (T) and paternal (C) alleles were already expressed in this calf, so a second liver biopsy was utilized from a 10-day old Holstein calf heterozygous for the exon 2 SNP (calf M446). This second calf expressed both alleles, however transcripts were expressed from the maternal allele at a lower level than from the paternal allele, as determined from quantitative measurement from agarose gels.

A loss of imprinting of *IGF2* has been found in humans and sheep as the animal ages. In sheep, by 6 months of age, the liver had lost its imprint and *IGF2* was being biallelically expressed while the kidney still maintained the imprint (McLaren and Montgomery 1999). Such a loss of imprinting has not been demonstrated in rodents possible due to the lack of an equivalent human P1 promoter (Ohlsen et al. 1994).

The unexpected result of the within-described studies indicates that the *IGF2* exon 2 SNP can be used to predict the REA size in cattle offspring. When the offspring obtain the variant allele from their mother, REA size is increased. Thus, the knowledge of the offspring's SNP pedigree is a predictor of REA size, in contrast to the traditional method of phenotypic sorting of animals based on frame size, which is not well correlated with REA size.

It is therefore an object of the present invention to provide a method of predicting the quality of a beef carcass through knowledge of the individual animal's genotype. It is a further object of the present invention to use the *IGF2* exon 2 SNP as a predictor of *IGF2* expression patterns which are known to result in an increased REA, and to use the knowledge of *IGF2* expression and genetic imprinting in breeding and herd management

applications. Finally, it is an object of the present invention to use the knowledge of an offspring's SNP pedigree to predict whether it has genetic potential from increased REA.

The observation that an SNP for *IGF2* exon 2 is correlated with increased REA size has implications for both the breeding and management throughout the various segments of the beef chain or cattle industry. Depending on which part of the chain the producer/company is involved, different management or breeding strategies will apply.

The breeding aspect of this SNP will ultimately increase the selection pressure that can be applied to selective mating. Since the presence of the SNP variant in the mother is phenotypically expressed in the offspring as an increase in REA size, producers will be able to make breeding/culling decisions on daughters of sires to select for the large-size REA trait. Firstly, the ability to select for C/C homozygous dams will ensure that REA size is maximized in offspring. Secondly, by assaying heterozygotes to determine whether their C allele came from their mother or father will likewise serve to permit the identification of cattle with maximal genetic potential for producing high value cuts of meat based on predicted REA size.

These types of selection are currently not possible, as a sire that exhibits a large-size REA phenotype may not produce offspring with the same large-size REA trait, due to the fact that the offspring express these traits based on the maternal side of their genetic makeup. Therefore, using the method of the present invention, a breeder will be able to select for daughters, which while not expressing the large-size REA trait, will pass on that trait to their offspring.

The present invention is also an improvement on the current method of sorting cattle according to parental phenotypes. As REA size is shown by the enclosed data to be predicted by SNP maternal genotype, more accurate breeding decisions will be possible by employing the method of the present invention. Knowledge of whether the offspring has received the SNP variant from its mother or father will also lead to more accurate breeding and management decisions. Since the critical factor in the eventual REA size in

offspring is determine by receiving the SNP variant from its mother, the method of the present invention will allow the selection or culling of offspring depending on whether they have the paternal or maternal SNP.

If a producer can intelligently modulate nutrition provided to animals during phase 1 and 2 of their growth, based on their genetic potential, then the economic return on the genetic potential can be maximized. Animals possessing various *IGF2* SNP genotypes will have different protein and energy requirements. Specifically, animals expressing the CC genotype, which is associated with increased REA size, can be expected to have higher protein requirements. Present feeding practices, where animals are not distinguished on the invention described herein, under-supply protein to "CC" animals and over-supply protein to "TT" animals. Using the method of the present invention therefore, producers will be able to maximize the genetic potential of individual animals.

The present invention will also be useful in the management strategies employed by feedlot operators. In the first two phases of the animal's life, which include skeletal and muscular development up to physiological maturity, the nutritional requirements, i.e. protein requirements needed to meet genetic potential for protein accretion, are based on pen basis, and not on knowledge of individual growth or protein production potential.

Current methods of sorting use anecdotal frame measurements, classifying animals as small, medium, or large-framed. At that point the genetic potential for all the animals in a pen or group are treated as the same. The present invention shows this method of sorting to be incorrect. Using the method of the present invention, sorting animals by their SNP genotype will allow the producer to group cattle according to genotype within a certain skeletal and muscular range. Thus, a precise method of sorting is made possible from which the environmental and nutritional parameters can be tailored to take full advantage of each individual animal's genetic potential for muscular, and REA size, development. Knowledge of each individual animal's genetic potential will ultimately increase production efficiencies.

Finally, the method of the present invention will allow those in the slaughter industry to better satisfy their requirements for end beef products, and to more accurately price the animals they purchase for processing. At present REA size is a proxy for high end cuts on the carcass. The greater the REA size, the more valuable the animal. Through application of the method of the present invention, slaughterhouses will know what "value" to expect of a carcass upon processing, based on the knowledge of its SNP pedigree. The processing industry will therefore be able to bid for animals more accurately than is currently possible using prior art methods of sorting based on frame size, as they will have knowledge of whether a carcass has a greater or lesser potential to yield higher value cuts of meat.

The foregoing is considered as illustrative only of the principles of the invention. Further, since numerous changes and modifications will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described. Accordingly, all such suitable changes or modifications in structure or operation that may be resorted to are intended to fall within the scope of the claimed invention.

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Claims.

1. A method of selecting livestock with improved qualities comprising identifying a single nucleotide polymorphism (SNP).
2. The method of claim 1, wherein the SNP is in exon 2 of *IGF2*.
3. The method of claim 2, wherein the SNP is associated with increased rib eye area (REA) production.
4. The method of claim 3, wherein the method further comprises using a primer pair comprising the sequence CCTCAGCCTCATCCCCTCCTTTGC and CTGTGCTCTATTTGCTGTGTTGTCT.
5. The method of claim 4, wherein the exon 2 SNP is as described in GenBank as accession #AY237543 and AY237544.
6. The method of claim 5, wherein the wherein the SNP is selected from the group C/C, C/T, and TT.

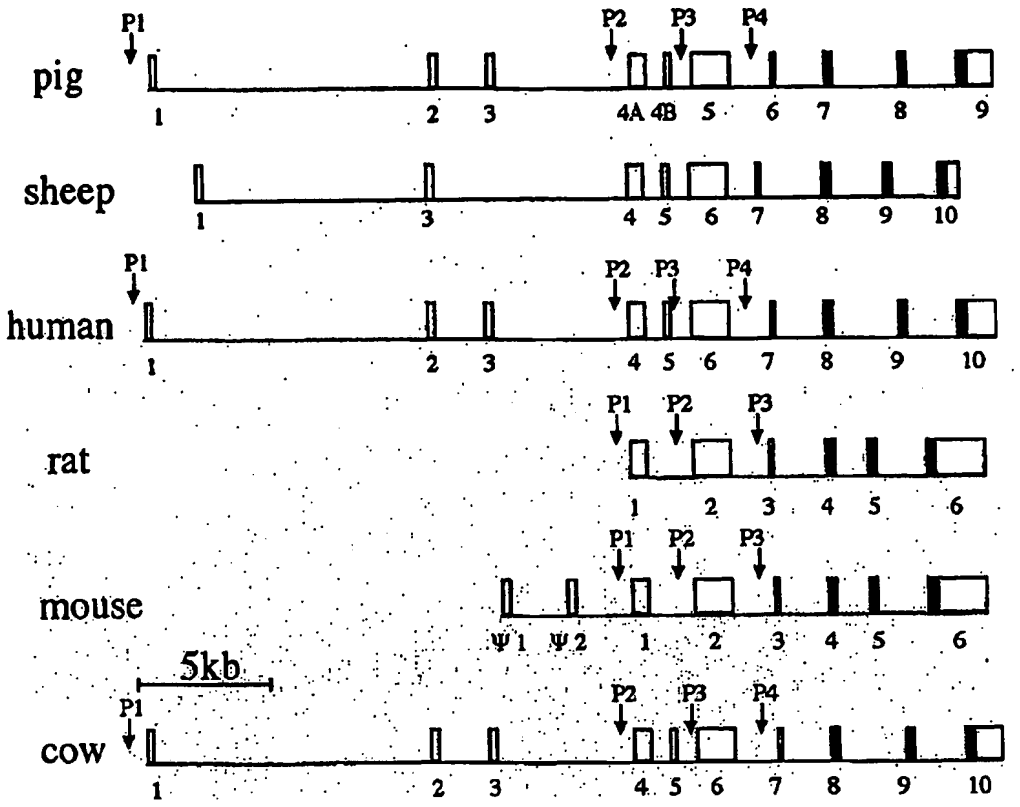

Figure 1. A comparison of the genomic organization of the pig, sheep, human, rat, mouse, and cow *IGF2* genes (Adapted from Ohlsen et al. 1994, Amarger et al. 2002). The numbered boxes represent the *IGF2* exons. Open and solid boxes indicate untranslated and translated exons respectively. ψ_1 and ψ_2 are the two-psedo exons identified in the mouse *IGF2* gene (Rotwein and Hall 1990). Promoters are indicated by P1 - P4.

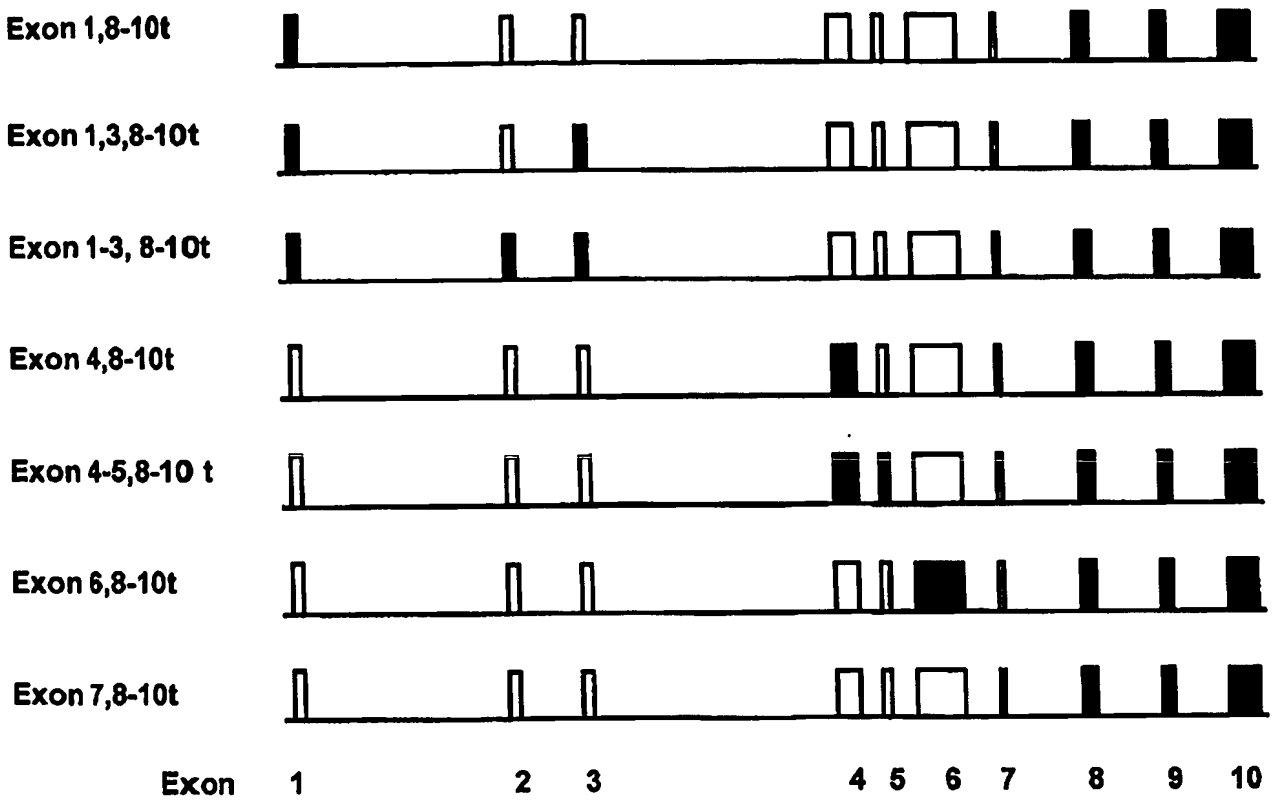

Figure 2. A gene structure comparison of the 7 different *IGF2* transcripts found in the different tissues of an 18 month-old steer. The black exons are the transcribed exons in each mRNA. Note that only exons 8-10 are translated into protein and are present in every transcript.

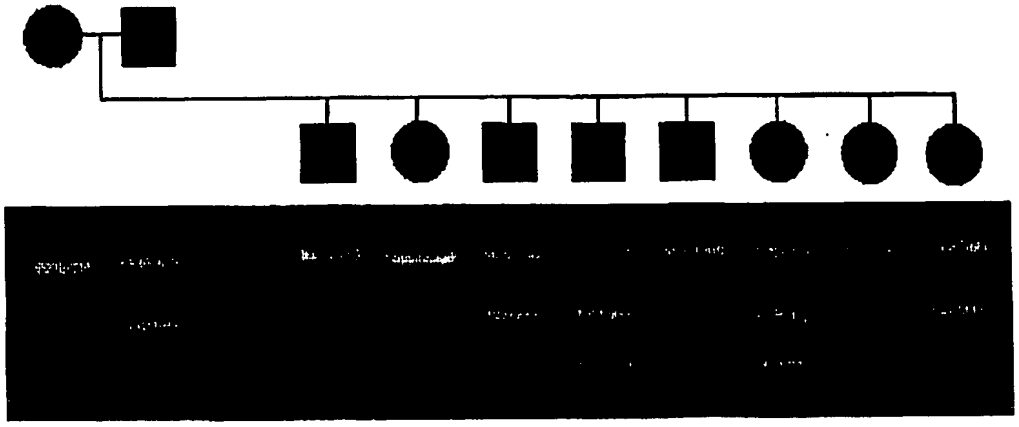

Figure 3. Photograph of an ethidium bromide stained agarose gel (3%) of an embryo transfer family from a heterozygous (C/T) Charolais sire and homozygous (C/C) Limousin dam. Four calves are heterozygous for the T allele and the other four calves are homozygous for the C allele. The C allele is 185 bp and the T allele is 118 and 68 bp. The 32 bp product is not shown.

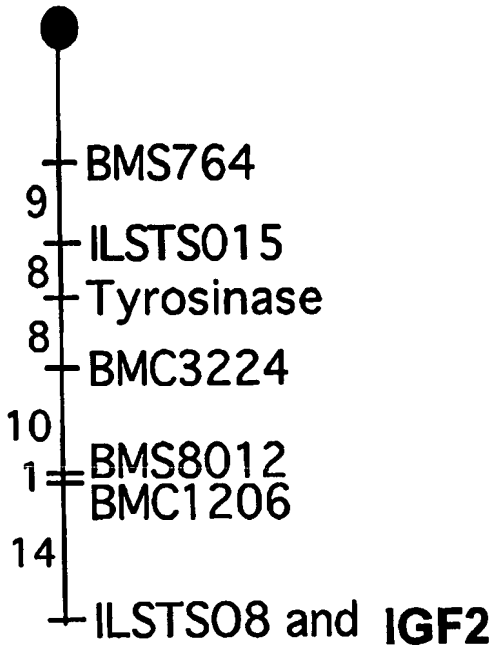

Figure 4. A linkage map showing the location of the microsatellites used to map IGF2 to the telomeric end of BTA 29.

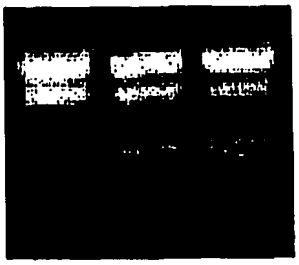

Figure 5. Photograph of an ethidium bromide stained agarose gel (3%) of a possible informative family for the exon 2 SNP. Calf M444 in lane 2 is heterozygous (C/T), as is his dam, cow 491 (C/T) in lane 3. For this family to be informative, the sire would have to be homozygous C/C as is the animal in lane 1 (or T/T) to assign parental origin to the calf's alleles.

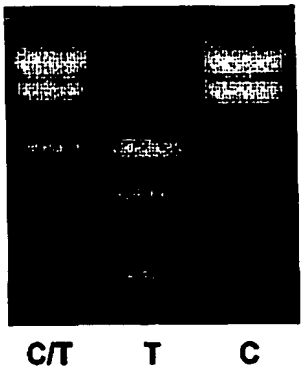

Figure 6. Photograph of an ethidium bromide stained agarose gel (3%) of the different alleles observed with the exon 2 SNP digested with *Bsr1*. To demonstrate genomic imprinting, fetal tissues would be harvested from a holstein calf heterozygous (C/T) for the exon 2 SNP. Expression analysis is expected to display expression of the paternal allelle. The genomic DNA would show heterozygousity (C/T) while the cDNA from the different tissues would express the paternal allele only (C or T). As the animal ages, biallelic expression (C/T) would be expected to reappear in the liver tissue but not in the kidney tissue (C or T).

Figure 7 - cDNA Sequences of IGF2 Gene Exons and Introns

IGF2 Sequence Obtained by Julie Goodall and Sheila Schmutz
July 21/03

Exon 1:
5' – TTCACGCAAGGTGGCCCTGGCGCGCAGGTCGGGGCAGGGGTGCCCCGCAGG
AGCTGAG - 3'

Exon 2:
5' - CCTCAGCCTCATCCCCTCCTTTGCCTCCCAGTCAAGCCTGCTGGGGGGTC
TGAGCACCACAGCCAGAGCACCCCCGCCTTGGCAGCGACTGCTACTATTG
GCCCAGCCAGCGGATCATCCACCTGGCAGTCGAGAGCCTGGGGCACCAGY
GACGTCCAGGTCCTCTTTACCCACCGCCCAGGGGAGCTTCAGAGACAACA
CAGCAAATAG AGCACAG- 3'
Where Y = C or T

Exon 3:
5' - GGGACGAAGAGTCACTGTTGACCTTGAGGACGAGGAGGTGGCCTTCAGCT
CCCAGCCCCAGGGCCCCACACCCAGGCCAGGTCAGCCCTTTGCCCAGGCT
GCCCCCCGGCCAGAACCCGCAGCAGGCCAGAAACCCCAGCCCGGAGGTCA
GCTGCTGTGACCTGTGGCCCTTGATCCCCACCTGCTCAGAATTGAGGCCC
GTGGCCCCAGCCCGCAGAG- 3'

Exon 4:
5' – GGCCCTTCCCGCGTCTGCTACGAAGTACCCGGAGCTCCTCGGATGCGGGA
AATTTCTCTCGTCCTCCTCACACACATTCGGTCGGGCAGCTTGTCCGCCA
GGACGTAGGCAGGGCTTCTCCCGCGTCCAGGAGAACGACTGGGCATTGCC
CCCAGTTTCCCCCAAATTTGGGCATTGTCCCCGGGTCTTCCAACGGACTG
GGCGTTGCCCCCGGACACTGGGGACTGCCCCGGGGTCTCGCTCACCTTCA
GTGCGCCTACCGCCCGCCGCAGATCGCTCGCTCGCTGCCTCGACTCCTCC
GCGCCCGCGGACGCAGCCTTCGGCCTCCAGCCTCGCG- 3'

Exon 5 :
5' – GAGATGGTTTCCCCAGACCCTCAAATGACCGTGGTGGCCCCCGGGGCTGA
ACCCGAATCTACGCAAGTCCAACGCATAGAGGACGGGGGAACCATTATCC
GGATATTTTGGGTGGGCCCCAAAGGCGAGCTGCTTAGACGCACCCCGGTG
AGCTCGGTCCTGCAG- 3'

Exon 6:
5' – TTTCAGAGCGGCGCTGGCAGAGGAGTGCCCGGCAGGAGGGCCTTCGCCCG
CTGTTCGGTTTGCAATACGCAGCAGGAAGGTGGGCGGCCCTGGTGCCGGCTTC
CAG - 3'

Exon 7:
5' – TCCTCGGAGGCAGCCTTCCAGACTCCTCCTCCTCCTCCTCCTCATCC
TCCTCCAGCCCCAGCGAGCCTCCTGTCCAGCTGCAG- 3'

Exon 8:
5' –ACATCAATGGGGATCACAGCAGGAAAGTCGGTGCTGGTGCTTCTTGCCTT
CTTGGCCTTCGCCTCGTGCTGCTATGCTGCTTACCGCCCCAGCGAGACTC
TGTGCGGCGGGGAGCTGGTGGACACCCTCCAGTTTGTCTGTTCGGACCGC
GGCTTCTACTTCAA- 3'

Intron 8:
5' – GCTGGCTGTCCTTGCAGGGCCACCAGGTGTCCCGTCCCGAGGGTGGGGGC
GGGGGCTGCCCGGAGGACCCCCRCCTCTGCCAGGTGCGGGCACTGACCTG
TCCTGCCCTTCCTCCCTGGGGGACCGCCAG - 3'
Where R = A or G

Exon 9:
5' – GCCGACCATCCAGCCGCATAAACCGACGCAGCCGTGGCATCGTGGAAGAG
TGTTGCTTCCGAAGCTGCGACCTGGCCCTGCTGGAGACTTACTGTGCCAC
CCCCGCCAAGTCCGAGAGGGATGTGTCTGCCTCTACGACCGTGCTTCCGA - 3'

Intron 9:
5' – GTAAGATGGCCCCCCAGCTCCGGCCCAGCCCCTGCGAGAGGAACCCTCCC
CTCTCTGCCCCTGCAGCCCCGCAAGGCTGTCACCCCAGAGCCAGGGGACC
AGGGCACAGCCAGCTGCTTAGCTGAGAGGGCAGCCAGGGAGTTCTTTACAC
CTGCCCTCTCCCCACTGGGGCACTGGCCAGCAGCGCGGGTGGGCGCTGGCC
TATCACACCTGCCATCGCACCCTGGGCACCTGGGGCAGCTTAGGCGTTGC
CCCCGTGTCCCCCGGACCCTGGGCAGCCCCTGACTGGCTCTTCCTCTCCC AG- 3'

Exon 10 and 3' UTR (untranslated region):
5' – GACGACTTCACAGCATACCCCGTGGGCAAGTTCTTCCAATCTGACACCTGGAAG
CAGTCCACCCAGCGCCTGCGCAGGGGCCTGCCCGCCTTCCTGCGAGCACGCCGGGG
TCGCACGCTCGCCAAGGAGCTGGAGGCGCTCAGAGAGGCCAAGAGTCACCGTCCGC
TGATCGCCCTGCCCACCCAGGACCCTGCCACCCACGGGGGCGCCTCTTCCAAGGCAT
CCAGCGATTAGAAGTGAGCCAAAGTGTCGTAATTCTGCCAAGTGGCACCATCTACCT
CGCGCCGACCTCCTGACCGGGACCGCCCCACTAGGTCTCTCTCTGAAATCCCTGTAC
CGTCCTGTCTGCGGGCTCCCCTGCCCCGGCCTCTGTGCCCCAACCTCCCCACGTCAG
GCGAATCCCCCTCGGCCCCCTCCATCTGGCTGAGGGGATCAGAACAACATCTCTAAA
AATGTACAAAACCAATTGGCTTTAAATATCCCCCCAAATTATCACCCCCCAAATTAC
CCCCAAATTACACAACCAAAATTGCAATCATGAACCCCTCAATCAGCCCCCTTGAAA
CGAATTGGCTTTTTAGCAACACCAGAAAAGCAAACTAGCTTTCCAAAAACTTCTTAA
AAAAAAAAAAATCAATTGGTTGAAAAAAATACTAAAAATAAATTGGCTTAAAAAAA
ATTGGG- 3'

Table 1. Sequenced exons and introns of the cattle *IGF2* gene to date.

Exons	Primers for exon	Sequence from CCA herd sires	Sequence from cDNA	Presence of SNP
1	No	No	Yes	Unknown
2	Yes	Yes	Yes	Yes
3	Yes	Yes	Yes	No
4	Yes	Yes	Yes	No
5	Yes	Yes	Yes	No
6	Maybe	No	Yes (3' end)	Unknown
7	Yes	No	Yes	Unknown
8	Yes	Yes	Yes	No
Intron 8 (3' end)	Yes	Yes	N/A	Yes
9	Yes	Yes	Yes	No
Intron 9	Yes	Yes	N/A	No
10	Yes	Yes	Yes	No
3' untranslated ends	Yes	Yes (Not great sequence)	N/A	Not likely

Table 2. The presence of the 7 different *IGF2* transcripts in each of the 15 different tissues from an 18 month-old steer as indicated by *. The transcription of the different transcripts is initiated by 1 of 4 different promoters (P1-P4).

	P1			P2		P3	P4
Tissues	Exons 1,8-10	Exons 1,3,8-10	Exons 1-3,8-10	Exons 4,8-10	Exons 4-5,8-10	Exons 6,8-10	Exons 7,8-10
Brain							(*)
Spinal Cord				(*)	(*)	(*)	(*)
Spleen							(*)
Abomassal Muscle							
Lung							
Lymph					(*)	(*)	(*)
Heart							
Kidney				*	*		*
Adrenal Gland					(*)		(*)
Rumen Wall							(*)
Liver	*	*	*	*	*	*	(*)
Thymus Gland				(*)	(*)		*
Muscle					(*)	*	(*)
Small Intestine							(*)
Abomassum					(*)		(*)

* Sequenced using ABI sequencer at PBI

(*) PCR product of corresponding size was obtained but not sequenced

Table 3. Results of genotyping several Greenbrae dairy herd offspring for the IGF2 exon 2 SNP for subsequent imprinting analysis.

Calf #	Lab #	Birth Date	Genotype	Dam #	Lab #	Genotype	Sire	Lab #	Genotype	Marlene	Other info	Kill Date
M440	03-029	Jan./03	C/T	435	03-040	C/C	Ourdream Blitzen	03-169	T	Sell	Informative	Apr.29/03
M441	03-030	Jan./03	C/C							Sell		
M442	03-031	Jan./03	T/T							Sell		
M443	03-026	Mar. 1/03	C/T							Sell	Twin	
M444	03-027	Mar. 14/03	C/T	491	03-032	C/T	Alta Shady	03-045	T/T	Keep	Informative	Apr.23/03
M445	03-028	Mar.19/03	C/C							Sell		
M446	03-167	May 5/03		410	03-168							

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/CA04/001039

International filing date: 20 July 2004 (20.07.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/489,026
Filing date: 22 July 2003 (22.07.2003)

Date of receipt at the International Bureau: 01 September 2004 (01.09.2004)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)